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Biosynthesis and characterization of zinc oxide nanoparticles using plant extracts of *Peperomia pellucida* L. and *Celosia argentea* L.

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Abstract
The present study investigated a simple ecofriendly approach for synthesis of zinc oxide (ZnO) nanoparticles using aqueous plant extracts of leafy vegetable plants *Peperomia pellucida* and *Celosia argentea*. The plant extracts were acts as reducing and capping agent, while Zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] was used as a precursor. The formation of ZnO nanoparticles were confirmed by visual observation followed by UV-Visible spectral analysis. The absorbance peaks for *P. pellucida* leaf, stem and roots was 299 nm, 299 nm and 293 nm. The absorption peaks located at 342 nm, 304 nm and 308 nm for leaf, stem and roots of *Celosia argentea* respectively. The results confirmed that these two herbs could be exploited for the production of valuable ZnO nanoparticles at room temperature, which can overcome the hazardous issues related to the chemical, physical and conventional methods of nanoparticles synthesis.

Keywords: Plant extracts, Zinc oxide nanoparticles, Reducing agent, Bioreduction, UV-Visible spectra.

1. Introduction

The biosynthesis of Zinc oxide (ZnO) nanoparticles gains significant credit with reference to their cost-effective, non-toxic, self-aggregation, antibacterial and gas sensing properties. ZnO nanoparticles play vital role in the synthesis of biological pigments, photo catalytic issues, production of piezoelectric devices, chemical sensors, drug carriers in targeted drug delivery mechanisms, and the production of cosmetics such as sunscreen lotions etc. Zinc oxide nanoparticles are employed in various biological and pharmacological applications due to its non-toxic nature. Zinc oxide efficiently protects broader UV range than other molecular UV-absorbers [1, 2].

Recently various parts of plants proved to be the potential reducing agents for production of nanoparticles [3]. Leaves [4], stem [5], fruits [6], flowers [7], seeds, barks and gums [8, 9] were employed for the synthesis of eco-friendly nontoxic nanoparticles with valuable biomedical properties. Plant based zinc oxide nanoparticles are widely accepted in cosmetic industry and agriculture [10, 11]. Plant extracts mediated ZnO nanoparticle synthesis have been reported in *Passiflora foetida* [12], *Coriandrum sativum, Acalypha indica* [13, 14], *Calotropis procera* [15] etc. The present study aims to synthesize Zinc oxide nanoparticles using two herbal leafy vegetables viz, *Peperomia pellucida* and *Celosia argentea*.

Peperomia pellucida L. is considered as epiphytic medicinal plant belongs to the family Piperaceae. It has the potential to evolve on nutrient rich woods, rocks and roofs [16]. It is commonly known as pepper elder, little heart, shining bush and silver bush, belongs to one of the largest genera of the family Piperaceae.

This annual herbaceous species is native and endemic to the Caribbean countries, characterized by succulent, angular trailing stems, shiny, heart-shaped and fleshy bright green leaves, single seeded fruits on several fruiting spikes with a mustard-like odor and usually reaches maximum 15 to 45 cm in height. It grows as a shrubby creeper epiphyte on rich habitats (Fig. 1). The aerial parts possess waxy surfaces [17]. It is mainly distributed in Central and South America, Africa, South-East Asian countries, and Australia [18, 19]. It prefers to grow in loose, humid soils and a tropical to subtropical climate, where it can be found wild on slightly shaded and damp areas such as brooks, walls, yards and even roofs, but widely naturalized and cultivated as a leafy vegetable [20].

The essential phytochemicals from *P. pellucida* are peperomins, sesamin, isoswertisin, flavonoids, phytosterols, arylpropanoids, saponins, terpenoids, isoquinolin, pellucidin, dillapiole [18, 21]. Leaves yield tannins with above said photochemicals with rich source of ash, crude fiber and carbohydrate content and minerals such as sodium, manganese, iron, zinc and copper. The major essential oil is hydroxylated sesquiterpenes, which consists of carotol, dillapiole and trans-caryophyllene [22].

Fig 1: *Peperomia pellucida* plants in pots.

Fig 2: *Celosia argentea* in natural conditions.

Celosia argentea L. (Amaranthaceae) is an important tropical leafy vegetable with a great medicinal value. It is herbaceous, erect and branched plant which bears simple and spirally arranged leaves, pinkish flowers, globular fruits and minute black seeds (Fig. 2). Roots are tuberous, white and cylindrical in shape [23]. It is commonly known as sunishannaka, safed murga, silver cock's comb, quail grass and wool-flower [24]. Genetic diversity of 16 populations of *C. argentea* was investigated using sequence-related amplified polymorphism [25]. It is used as leafy vegetable along with seventy different species of *Celosia* [26]. *C. argentea* plant is common in West Africa, from Sierra Leone to Nigeria. It is cultivated as leafy vegetable in the rainforest zone of Nigeria, Benin, Cameroon, Gabon, and Togo. It is distributed throughout India, Sri Lanka, Yemen, Indonesia, America and West indies [27].

Traditionally *C. argentea* is used for the treatment of diarrhea, piles, bleeding nose, disinfectant, inflammation, uterine diseases, diabetes mellitus, rheumatoid arthritis, gynecological disorders [28-30] and as an aphrodisiac [31, 32]. The flowers are used against snakebite [33]. It is endowed with benzoic acid derivatives, stilbenes, tannins, lignans, anthocyanins, flavonoids and coumarins, flavonoids, diterpenes, steroidal saponins, celocsin A,B,C and D [34-36]. Literature reveals that *C. argentea* reported to possess wound healing [37], immunological [38], anti-inflammatory [39], anti-cancer, antioxidant [40], hepatoprotective [41], antimitotic [42], antispasmodic, diuretic, antipyretic [43], antibacterial and anti-diabetic properties [45].

The eco-friendly processes are increasingly aided in chemical technologies and requires at upsurge rate worldwide for environmental issues [45]. This is the first attempt to synthesize biologically hazard-less Zinc oxide nanoparticles from the various aqueous extracts of *P. pellucida* and *C. argentea*.

2. Materials and Methods

2.1. Collection of plant materials

Peperomia pellucida and *Celosia argentea* are mostly cultivated as food crop and as ornamental plants. The plant materials were collected from the Institute campus in month of January to March, 2015 and identified with the help of 'The Flora of Presidency of Madras' [46]. The whole plant was harvested to get fresh green leaves, stems and roots.

2.2. Preparation of plant extracts

The plant extracts (broth solution) were prepared to prepare zinc oxide nanoparticles on the basis of ecofriendly measures, time and cost effectiveness. The plant materials were thoroughly cleaned with running tap water to remove debris and other contaminations, followed by double distilled water and air dried at room temperature. All the parts were separated and finely chopped into small pieces (Figs. 3AB-8AB). About 5 gm of finely cut leaves, stems and roots were kept in a 250 ml Erlenmeyer flask with 50 ml of sterile double distilled water and then the mixture was boiled for 30 min. The aqueous plant extracts were cooled down and filtered by standard filtration method with Whatman filter paper no.1 and stored at 4 ^{o}C in refrigerator for further experimentation.

2.3. Preparation of precursor

Zinc Nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] (Merck, Mumbai) was used as a precursor to synthesize ZnO nanoparticles in the present study. 1mM Zinc nitrate solution was prepared using sterile double distilled water and stored in brown bottle at 4 ^{o}C for further use to synthesize ZnO nanoparticles from leaf, stems and roots of *P. pellucida* and *C. argentea*.

2.4. Synthesis of ZnO nanoparticles

Three boiling tubes were used in each experiment to synthesize ZnO nanoparticles, one contained 10 ml of 1mM Zinc nitrate solution and the second one contained 10 ml of aqueous plant extract and the third one contained 9 ml of 1 mM Zinc nitrate solution and 1 ml of plant extracts as test solution. The reaction mixture from the third tube was centrifuged at 5000 rpm for 15 min to obtain the pellet after 2 to 3 hrs. Supernatant is discarded and the pellet is dissolved in deionized water. This setup was incubated in a dark chamber to minimize photo-activation of zinc nitrate at room temperature. Reduction of zinc nitrate to zinc oxide was confirmed by the color change of solution from colorless to light yellow. Nanoparticles formation was also confirmed by using UV–Visible spectroscopy.

2.5. UV-VIS spectra analysis

The bioreduction of ZnO metal ions in the mixture solutions was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VIS spectal analysis of the sample was done by using Systronics Double Beam spectrophotometer (Model 2202, Systronics Ltd.) at room temperature operated at a resolution of 1 nm between 200 nm and 700 nm. One milliliter of the sample was pipetted into a cuvette and analyzed at room temperature.

3. Results and discussion

This method explains the simple plant extract-mediated bioreduction of Zinc oxide nanoparticles involves mixing the aqueous plant extracts with an aqueous solution of the Zinc nitrate metal salt. The synthesis of ZnO nanoparticles achieved at room temperature and completes within few minutes.

3.1. Visual observations of ZnO nanoparticles synthesis

In all experiments, addition of plant extracts of *P. pellucida* into the test tube containing aqueous solution of Zinc nitrate led to the change in the color of the reaction solution to yellowish (Figs. 3C-5C). The leaf extracts of *C. argentea* changed in to yellow when challenged with 9 ml of 1mM Zinc nitrate solution (Fig. 6C). On addition of stem and root

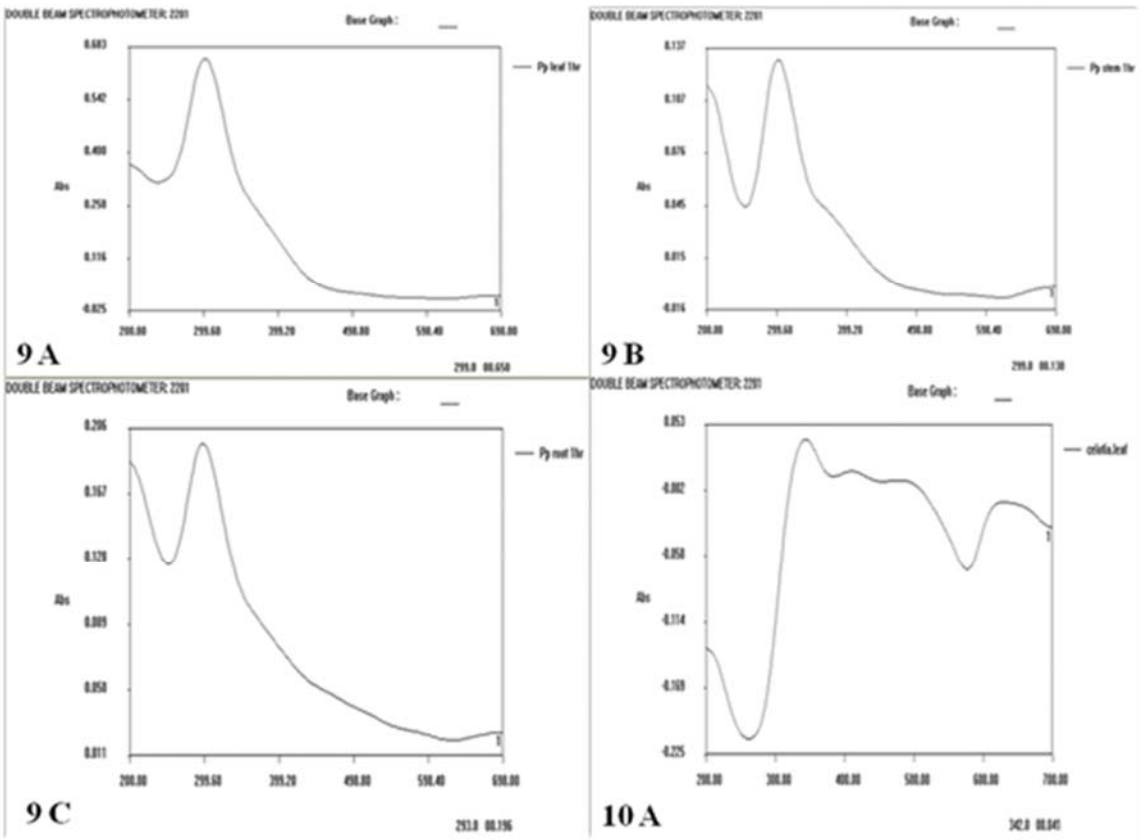
extracts of *C. argentea* to the aqueous Zinc nitrate solution kept at room temperature the color of the solution changed from faint light yellow but it was nosst impressive like other extracts (Fig. 7 C-8C). It was changed into yellowish when the reaction mixture was heat up for 15 min in a water bath indicated the formation of Zinc oxide nanoparticles. The time taken for the reaction mixture to change color was varied with plant extracts. The color change of reaction mixtures within reaction duration was due to excitation of surface plasmon vibrations in nanoparticles [47].

Figs 3 to 5: Different parts of the plants used for biosynthesis of ZnO nanoparticles. 3(A to C) Leaves and the reaction mixtures of *P. pellucida*, 4(A to C) Stem segments and the reaction mixtures, 5(A to C) Roots and the reaction mixtures.

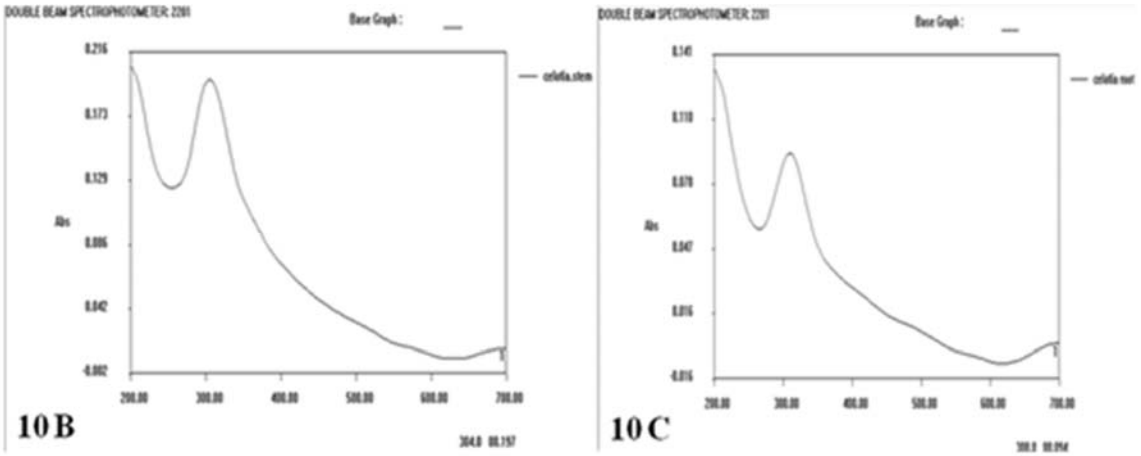
Figs 6 to 8: 6(A to C) Leaves and the reaction mixtures of *C. argentea*, 7(A to C) Stem and the reaction mixtures, 8(A to C) Roots and the reaction mixtures.

3.2. UV–Vis spectral analysis

Zinc oxide nanoparticles were synthesized using different extracts such as leaf, stem and roots of *C. argentea* and *P. pellucida*. The UV- Visible spectra of plasmon resonance band was observed at 293-342 nm similar to those reported in *Lawsonia inermis* [48], *Duranta erecta* [49] and *Melia azedarach* [50]. The aqueous reaction mixture of *P. pellucida* leaf and stem showed strong absorption peak at 299 nm, and the root mixture showed at 293 nm (Figs. 9A-C). The aqueous reaction mixture of *C. argentea* leaf showed strong broad peak at 342 nm, stem and root peaks located at 304 nm and 308 nm respectively (Fig. 10A-C). The resulted absorptions range was close to the chemically prepared Zinc oxide nanoparticles at 358 nm [1].



Figs 9: UV-Vis spectral analysis of Zinc oxide nanoparticles. (9A) Absorbance peak with leaf extract, (9B) Absorbance peak with stem extract, (9C) Absorbance peak with root extract of *P. pellucida*



Figs 10: (10A) Absorbance peak with leaves extract of *C. argentea*, (10B) Absorbance peak with stem extract, (10C) Absorbance peak with root extract.

There were decreased wavelength observations from leaf to root mixtures of both the plant extracts (Table 1). Tripathy *et al*. [51] reported that the slight variations in the values of absorbance are due to the changes in the particle size. With the same concentrations of leaf and stem extracts of *P. pellucida*, there was no change in absorbance values. The leaf extracts of *C. argentea* as well as *P. pellucida* showed an increase in intensity of absorption. The UV-Visible spectra recorded after different time intervals and the extracts of *P. pellucida* have been completed within 1 h. The initiation of reaction was varied with the plant extracts of *C. argentea*. UV-Visible spectroscopy was also used to study the size and shape of controlled nanoparticles in aqueous suspensions [52].

Table 1: UV-Visible absorption specta of Zinc oxide nanoparticles synthesized using *P. pellucida* and *C. argentea* aqueous extracts.

Sl. No	*P. pellucida* Reaction mixtures	*C. argentea* Reaction mixtures	UV-Vis absorption spectrum (nm)
1.	Leaf extracts	-	299
2.	Stem extracts	-	299
3.	Root extracts	-	293
4.	-	Leaf extracts	342
5.	-	Stem extracts	304
6.	-	Root extracts	308

The appearance of the yellow color at room temperature and raised temperature of plant extracts with Zinc nitrate precursor was due to the excitation of the Surface Plasmon Resonance (SPR), typical of Zinc oxide nanoparticles having absorbance values which were reported earlier in the visible range of 290-340 nm [48, 1, 53]. The increased intensity of absorption peaks of leaf extracts at room temperature and the color intensity with the duration of incubation were reported in this study. The results are agreed with the available reports and showing close results in absorbance of Zinc oxide nanoparticles synthesized by the extracts of *Acalypha indica* [14], *Passiflora foetida* [12] and *Hemidesmus indicus* [54].

The resulted UV-Visible spectral analysis revealed that the most rapid bioreduction of Zinc oxide nanoparticles were achieved using the plant extracts of *P. pelludica* and *C. argentea* as reducing agents. The visual observation implied the formation of Zinc oxide nanoparticles within 10 min from the extracts of *P. pellucida* and leaf extract of *C. argentea*, 40 min from the stem and root extracts of *C. argentea*.

4. Conclusion

A simple one-step green synthesis of stable Zinc oxide nanoparticles using *P. pellucida* and *C. argentea* plant extracts at room temperature was reported in this study. Efficient synthesis of stable ZnO nanoparticles was achieved within short duration of time. It proved to be a cost and time effective, eco-friendly, green approach for the synthesis of medicinally and industrially important ZnO nanoparticles. This green chemical reaction pathway could be a competitive alternative to the conventional methods explored for human health and environment.

5. References

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